



## Floral biology and reproductive attributes of six cultivated accessions of pigeonpea

Divya Mohanty<sup>1</sup>, Atika Chandra<sup>2</sup> and Rajesh Tandon<sup>1,\*</sup>

<sup>1</sup>Department of Botany, University of Delhi, Delhi- 110 007, India

<sup>2</sup>Department of Botany, Maitreyi College, University of Delhi, Delhi-110021, India

\*e-mail : rjtnd@rediffmail.com

Received: 20.09.2015; Revised: 14.12.2015; Accepted and published on line: 01.07.2016

### ABSTRACT

Leguminous crops offer numerous challenges in their improvement of yield. The narrow genetic base of present day legume cultivars is considered to be the major hurdle. Wide hybridization involving various cultivars and the relatives of target crop is considered a suitable strategy provided that there is scope for outcrossing, either naturally (dichogamy/herkogamy) or by manually overcoming the crossability barriers. Thus, the basic details of floral biology of crop plants in their respective agroclimatic zones are essential. Here, we provide a baseline data on the floral biology and some reproductive attributes of the six widely cultivated varieties of *Cajanus cajan* (pigeonpea). The study shows that the cultivars are predominantly protandrous, viability of the fresh pollen ranges between 60-90%, the potential brood size is realized by the time anthesis is achieved, variable rates of seed abortion (~ 8-26%) occur in the cultivars and pollen:ovule ratio suggests facultative xenogamous type of breeding system. The findings would be useful in designing pollination experiments to facilitate crosses among the cultivars and related species.

**Keywords:** legumes, protandry, maturity groups, tripping, seed abortion.

Pigeonpea, an important grain legume and a member of the 'millettioid' clade or 'warm-season/ tropical legumes', belongs to the third largest family of flowering plants. Apart from its high adaptability to cropping systems, it complements cereals and is a chief source of dietary proteins especially in the developing world. Globally the sixth most important food legume (Varshney *et al.* 2012), pigeonpea is increasingly grown in the semi-arid tropics, and India is the largest producer (Wani *et al.* 2009, Taggar and Singh 2015). In spite of the recent efforts made in breeding programs, the past 50 years has witnessed a very marginal increase in the productivity of the crop and that too due to the increase in land area under cultivation (FAO 2012). Production of the crop has stagnated due to both biotic and abiotic stresses faced during vegetative growth and poor tractability (Grover and Pental 2003, Barros *et al.* 2014). Once considered an 'orphan' crop, the pigeonpea genome has been recently decoded thereby making way for genomics assisted breeding (Varshney *et al.* 2005, 2012, Mir *et al.* 2014). However, the present day cultivars of pigeonpea display a narrow genetic base, accrued largely to origin and domestication (Varshney *et al.* 2013). The limited arable land further contributes an ever increasing gap between the potential and the realized yields of the crop.

Continuous efforts are needed to combine new technologies with conventional breeding approach to ameliorate the production and meet the demand. The breakthrough for increasing the harvest index can be achieved in two ways, either by exploiting heterotic breeding and widening the gene pool (Saxena 2008, Patel and Tikka 2014) and genetic manipulation to restructure the genome altogether (Lakshmi Sita and Venkatachalam 2008).

The papilionaceous flower of the crop, the predominant protogynous nature and anther dehiscence prior to anthesis are

believed to facilitate selfing in the species (Krauss 1932, Prasad *et al.* 1977). There are also reports on pre-anthesis self-pollination or chasmogamic selfing in the species (Frankel and Galun 1977). However, selfing rate among the cultivars vary and natural crossing does occur which may range widely from 5-70% (Wilsie and Takahashi 1934, Abrams 1967, Purseglove 1968, Bhatia *et al.* 1981, Kumaraswamy and Bawa 1989, Saxena *et al.* 1990, 1996, Sivaram and Jayaramappa 2013). In nature, many insect species especially those belonging to *Apis*, *Chalicodoma*, *Megachile*, *Ceratina* and *Xylocopa* have been recorded to bring about cross-pollination in pigeonpea (Williams 1977, Williams 1980, Brar 1992, Upadhyay *et al.* 1997, Abrol 2012, Singh *et al.* 2015). The natural outbreeding behavior of the plant is a very important attribute paving way towards its improvement (Saxena 2008). Based on this, some hybrid seed production systems have been developed in pigeonpea (El Baradi 1978, Saxena *et al.* 1992, Mallikarjuna and Saxena 2002, Saxena *et al.* 2005, Mallikarjuna *et al.* 2006, Srivastava *et al.* 2012, Zheng-Hong *et al.* 2012, Patel and Tikka 2014). Outcrossing is also believed to increase fruit and seed-set in the crop (Sivaram and Jayaramappa 2013). These findings suggest that crosses between the cultivars have proved to be a useful strategy to widen the genetic base. In doing so, it would be necessary to have baseline data on the floral biology of cultivars. In the present work we investigated some of the features of floral biology of six cultivars of pigeonpea.

### MATERIALS AND METHODS

Seeds of six cultivated accessions of *Cajanus cajan* (pigeonpea) were procured (Table 1) from the Genebank, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Most of the selected cultivars are suitable to grow under north Indian conditions

including that of Delhi. All the accessions were raised and maintained under natural conditions at the experimental plots of the University of Delhi located at Bawana, Delhi (28 °44' 402" N, 77 °07' 241" W). Seeds were sown during June in a freshly prepared field, fertilized with Urea (5 kg/sqft), Diammonium Phosphate (5 kg/sqft) and Potash (10 kg/sqft). The flowers were used for comparative study on the floral biology of the cultivars.

Four stages (stages 1-4) for each cultivar were identified as follows - bud with 1/3rd petal and 2/3rd sepal length (stage 1), mature bud with equal sepal and petal length (stage 2), mature unopened flower with 2/3rd petal and 1/3rd sepal (stage 3), mature open flower at anthesis (stage 4). Some of the characteristic reproductive features (descriptors) identified for the crop were also noted down according to the guidelines (Rao and Bramel 2000).

The average number of flowers borne by a cultivar was recorded from the randomly marked plants ( $n = 10$ ) of each variety during the peak period of their flowering. The flower production was computed as the multiplication product of the mean number of (i) floral buds per inflorescence; (ii) inflorescence per branch; and (iii) branches per plant.

For determining the pollen production, mature and undehisced anthers of each cultivar ( $n = 10$  flowers each) were released in 50 $\mu$ l of 70% ethanol containing Triton-X. For each cultivar, a known dilution was placed on the grid of a haemocytometer and number of pollen grains was counted. Total number of pollen grains in a flower was computed as the multiplication product of pollen produced per anther and the total number of anthers in a flower. The presence of starch in the pollen were localized by Iodine-Potassium iodide (I-KI) or Lugol (Johansen 1940), proteins with coomassie brilliant blue R (CBBR) (Dafni *et al.* 2005) and lipids with Sudan Black B (Baker 1947).

The fertility of the pollen grains was assessed ( $n = 20$  flowers, each cultivar) with 1% acetocarmine (Shivanna and Rangaswamy 1992). The viability of pollen was ascertained by the Fluorochromatic Reaction (FCR) test (Heslop-Harrison and Heslop-Harrison 1970). The preparations were viewed under the Fluorescein Isothiocyanate filter (FITC; UV excitation filter range of 450-490 nm) of an epifluorescence microscope (Carl Zeiss, Axio Scope A1).

Stigma receptivity was ascertained by localizing the activity of nonspecific esterases (Pearse 1972). The total number of ovules ( $n = 20$  flowers each) and ovule receptivity at different floral stages (stage 1- 4;  $n = 20$  flowers for each cultivar) was recorded for each cultivar (Sengupta and Tandon 2010).

In order to determine the natural fruit-set, inflorescences were tagged in randomly selected plants and monitored for the amount of fruits produced. The total number of seeds, number of seeds formed and the number of aborted seeds were counted

from the fully formed pods ( $n = 20$ ) of each cultivar for computing the abortion rate.

All the data collected were recorded in Microsoft Excel (Microsoft Office™ 2010) and mean and standard error were computed. Wherever relevant, data was analysed for its normal distribution through Shaipro-Wilk test and compared by employing suitable statistical test (ANOVA/ MANOVA) using SPSS v. 22 (IBM-SPSS Amos™ 22 IBM Corp 2013) and further analysed by Tukey's HSD test for homoscedasticity, as the variables were more than two.

## RESULTS AND DISCUSSION

Higher flower production in an inflorescence and in a plant increases the visual attraction for pollinators and promotes outbreeding (Thomson 1988, Klinkhamer & de Jong 1990, Kudo & Harder 2005). The pollination syndrome of pigeonpea appears to be suited for bee pollination, as many reports indicate visits of *Apis* spp on its various cultivars (Abrol 2012). Nectar is produced in trace amounts in the cultivars investigated (present work). There was a significant difference in the average flower production in an inflorescence amongst the six cultivars (Table 2). In this context cv. Asha, Upas 120 and Pusa-33 are high flower bearing cultivars, although the number of flowers borne in an inflorescence is higher in Pusa Ageti. The latter variety happens to be a dwarf cultivar with determinate inflorescence.

The flowering period of the cultivars (Table 3) showed that the selected cultivars belonged to five maturity groups (Singh *et al.* 2013). The longer vegetative growth phase of the long-medium duration varieties leads to inefficient land use and poor productivity than the shorter duration varieties (Singh 1996, Srivastava *et al.* 2012). This is largely due to longer exposure to stressed environmental conditions. In this context, the number of days to achieve 50% of the flowering was maximum in the cv. Bahar (141-160 days) and cv. Asha (121-130 days) and are categorized as medium-long and medium duration variety, respectively (Vales *et al.* 2012, Singh *et al.* 2013). Whereas, the cultivars T-21 (81-90 days), Pusa Ageti (81-90 days), Upas (71-80 days) are short duration variety and Pusa-33 (61-70 days) is extra-short duration variety (Vales *et al.* 2012, Singh *et al.* 2013). The shorter flowering duration varieties are better suited for sustainable land utilization in the crop intensification programmes (Srivastava *et al.* 2012).

In all the cultivars, the anthers dehisced longitudinally at Stage 2 (bud with equal petal and sepal length). The cv. Asha produced significantly greater amount of pollen grains ( $8440 \pm 201.98$ ) per flower than the other cultivars ( $F_{(5,94)} = 10.22, P = 0.001$ ; one-way ANOVA; Table 7). Rest of the five cultivars did not show any difference in pollen production (Tukey's HSD) viz., T-21 ( $7530 \pm 149.58$ ), Upas 120 ( $7150 \pm 76.88$ ),

PusaAgeti (6970 ± 208.19), Pusa-33 (7060 ± 192.59) and Bahar (7110 ± 132.83).

More than 90% of the pollen grains produced in each of the cultivars were normal and viable. Leguminous pollen grains are generally long-lived, as they may retain viability up to 5-6

days. Thus, if the pollen could be stored under suitable conditions, they would be amenable for transportation to aid in assisted breeding. There was a significant difference in the per cent pollen viability among the six cultivars (Wilks'  $\lambda = 0.471$ ,  $F_{(10,26)} = 9.402$ ,  $P = 0.001$ , MANOVA, Table 4; Fig. 1), while per

Table 1—Germplasm details of the six *Cajanus cajan* cultivars procured from ICRISAT and used in the present study (Source: Sharma, 1961; Singh et al., 2013; Singh et al., 2014).

Cultivar	ICP No.	Code	Pedigree	Year of release and the state	Area of adaptation	Indian states under cultivation
Asha	14722	CAS	C11 X ICPL 6	1993 (ICRISAT)	Central Zone, South Zone	Orissa, Maharashtra, Karnataka
Type-21(T-21) Upas 120	26 6971	CT2 CUP	T1 X T-190 Selection from P-4758	1961 (UP) 1976 (UP)	- Uttar Pradesh	Punjab, TN, UP Orissa, MP, UP, Maharashtra, Rajasthan, Punjab
Pusa Ageti	28	CPA	Brazil-1 x NP69	1971	Throughout India	Delhi, Haryana, Gujarat, JK, Kerala Karnataka
Pusa-33	15590	CP3	C11 X UPAS120	1988 (Delhi)	North West Plain Zone, Central Zone	Delhi, Haryana
Bahar	7197	CBA	Selection from landraces of Tirhut region	1986 (Bihar)	Bihar, Uttar Pradesh	West Bengal, North Eastern state, Assam, Bihar

Table 2- The mean flowers per inflorescence and mean flowers per plant across the six cultivars.

Cultivars	Mean flowers per inflorescence	Mean flowers per plant
Asha	8.43 ± 0.35 <sup>a</sup>	10,806.85 ± 670.14 <sup>b</sup>
T-21	7.8 ± 0.23 <sup>ab</sup>	7,332.85 ± 346.12 <sup>ab</sup>
Upas 120	9.02 ± 0.19 <sup>a</sup>	10,124.28 ± 773.03 <sup>b</sup>
Pusa Ageti	10.68 ± 0.41 <sup>bc</sup>	9,002.21 ± 432.88 <sup>ab</sup>
Pusa-33	8.82 ± 0.21 <sup>ab</sup>	9,352.63 ± 425.39 <sup>b</sup>
Bahar	9.5 ± 0.29 <sup>c</sup>	6,308.75 ± 218.16 <sup>a</sup>
F	6.17	10.98
P	0.001	0.001

a, b, c are the homogenous subsets of the Tukey's HSD test.

df = 5,54 for both mean flowers per inflorescence and mean flowers per plant

Table 3- Some descriptors of the six cultivated varieties.

Cultivar	Time to maturity (days)	Days to 50% Flowering (days)	Maturity group	Flowering pattern <sup>a</sup>	Flower color <sup>b</sup>	Streak color <sup>c</sup>	Streak pattern <sup>d</sup>	Pod color <sup>e</sup>	Seed color pattern <sup>f</sup>	Primary seed color <sup>g</sup>	Weight of 100 seeds (gms)
Asha	160-170	121-130	Medium duration variety (VI)	NDT	Y	R	MS	M	P	RB	12.58
T-21	150-170	81-90	Short duration variety (III)	NDT	Y	R	FS	M	P	RB	9.75
Upas 120	125-150	71-80	Short duration variety (II)	NDT	Y	R	FS	M	P	RB	8.88
Pusa Ageti	150-160	81-90	Short duration variety (III)	DT	Y	R	FS	M	P	RB	9.87
Pusa-33	120-140	61-70	Extra Short duration variety (I)	NDT	Y	R	MS	M	P	RB	7.16
Bahar	230-250	141-160	Medium long duration variety (VIII)	NDT	Y	R	MS	M	P	RB	10.94

a: DT-Determinate, NDT-Indeterminate, SDT- Semi determinate; b: I- Ivory, L- Light yellow, OY- Orange yellow, Y- Yellow; c: NO- None, Pu- Purple, R- Red; d: FS- Few streaks/ MS- Medium streaks/ DS- Dense streaks/ P- Plain, uniform coverage/ NO- None; e: DP- Dark purple/ G- Green/ M- Mixed green and purple/ P- Purple; f: P- Plain/ M- Mottled/ S- Speckled/ MS- Mottled and speckled/ R- Ringed; g: W- White/ BL- Black/ C- Cream/ O- Orange/ G- Grey/ P- Purple/ DP- Dark Purple/ LB- Light brown/ LC- Light cream/ LG- Light grey/ RB- Reddish brown

Table 4—Tests of between-subjects effects for the pollen fertility and viability across the six cultivars.

Source	Dependent variable (%)	Sum of squares	df	F	P
Cultivars	Pollen Viability	1.391	5,104	19.990	0.001
	Pollen Fertility	0.109	5,104	1.686	0.144

Table 5- Tests of between-subjects effects for the total number of ovules, total number of seeds and number of aborted seeds in the six cultivars.

Source	Dependent variable	Sum of squares	df	F	P
Cultivars	Total number of ovules	15.467	5,114	3.093	0.001
	Number of mature seeds	16.300	5,114	3.260	0.004
	Number of aborted seeds	1.945	5,114	0.389	0.032

Table 6- Natural fruit-set per inflorescence, fruit to flower ratio in six cultivars of *Cajanus cajan*.

Cultivar	Fruit-set per inflorescence	Fruit : Flower ratio
Asha	2.29 ± 0.11 <sup>a</sup>	0.27
T-21	2.96 ± 0.14 <sup>a</sup>	0.33
Upas 120	2.75 ± 0.16 <sup>a</sup>	0.31
PusaAgeti	5.85 ± 0.22 <sup>c</sup>	0.55
Pusa-33	4.19 ± 0.19 <sup>b</sup>	0.47
Bahar	3.86 ± 0.11 <sup>b</sup>	0.40

a, b, c are the homogenous subsets of the Tukey's HSD test.

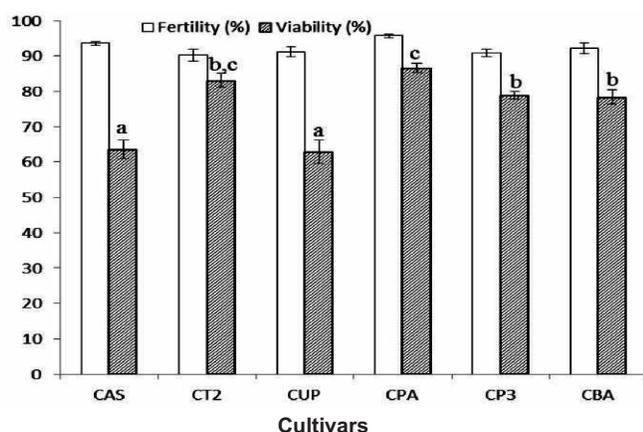


Fig. 1—Per cent viability and fertility of pollen grains from freshly dehisced anthers in the six cultivars of *Cajanus cajan*. The common letters indicate homogenous subsets. According to multivariate analysis there is significant difference ( $p = 0.001$ ) in the percent pollen viability among the varieties while percent pollen fertility is similar across the cultivars.

cent pollen fertility was similar (Table 4; Fig. 1). The cv. Upas 120 showed the least number of viable pollen grains and Pusa Ageti the most. Barring the two cultivars (Asha and Upas 120), the viability of fresh pollen in other cultivars matched the fertility. In cv. Asha and Upas 120, the tendency to rapidly lose pollen viability could be attributed to changes in relative humidity and ambient temperature after anther dehiscence (Aronne 1999, Devasirvatham *et al.* 2014). Loss in viability also correlated with the prolonged duration of exposure and desiccation in species where anther dehiscence occurred before the opening of flower (Pacini 1992, Nepi and Pacini 1993).

The freshly released pollen showed the presence of starch and lipids. The lipids could be demarcated only along the boundary by the deep black stained pollen wall. The blue

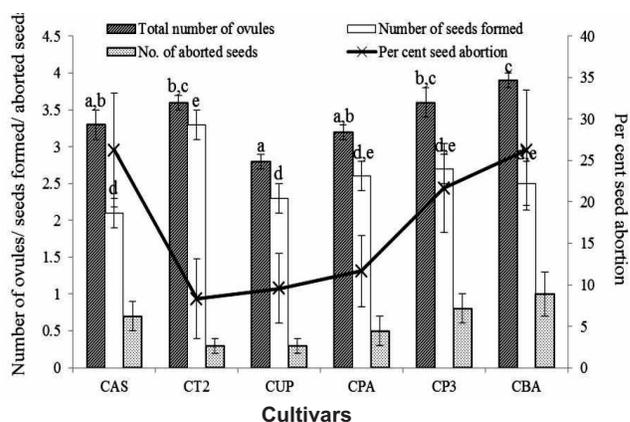


Fig. 2—Total number of ovules, number of seeds formed, number of aborted seeds and per cent seed abortion in the six cultivars of *Cajanus cajan*. The common letters indicate homogenous subsets. According to multivariate analysis there is significant difference in the total number of ovules ( $p = 0.001$ ) and number of mature seeds ( $p = 0.004$ ) formed among the varieties while seed abortion is similar across the cultivars.

colour and deep brown stained pollen grains indicated the presence of proteins and starch, respectively.

The onset of stigma and ovule receptivity was correlated in each of the cultivar (Table 7). Except in cv. Asha, anther dehiscence (stage 2) in the remaining cultivars began prior to the onset of stigma receptivity. This suggests that these five cultivars are protandrous and would require emasculation, preferably at stage 1 or 2, for cross-pollination experiments. This is in contrast to the many of the other cultivars of pigeonpea that are protogynous (Luo *et al.* 2009, Choudhary *et al.* 2012).

The extent of receptive ovules in an ovary is an indication of the potential brood size (seed number per fruit), provided

Table 7—A comparison of the key reproductive attributes between the six cultivars of pigeon pea.

Morphological appearance	S.No.	Aspect	Asha	T-21	Upas-120	Pusa Ageti	Pusa-33	Bahar
Mature flower with fully open banner petal (Stage 4)	1.	Pollen number	8440 ± 201.98	7530 ± 149.58	7150 ± 76.88	6970 ± 208.19	7060 ± 192.59	7110 ± 132.83
	2.	Pollen fertility (%)	93.7 ± 0.5	90.3 ± 1.7	91.2 ± 1.4	95.8 ± 0.5	91.0 ± 1.1	92.2 ± 1.5
	3.	Pollen viability (%)	63.5 ± 2.7	83.2 ± 1.9	62.9 ± 3.4	86.6 ± 1.4	78.9 ± 1.2	78.4 ± 2.1
	4.	Pollen histochemistry						
	a.	Starch	+	+	+	+	+	+
	b.	Proteins	+	+	+	+	+	+
	c.	Lipids	+	+	+	+	+	+
	5.	Receptivity of Stigma and Ovules at different stages						
a.	Bud with 1/3 <sup>rd</sup> petal and 2/3 <sup>rd</sup> sepal length (Stage 1)	Stigma	-	-	-	-	-	-
		Ovule	-	-	-	-	-	-
b.	Bud with equal petal and sepal length (Stage 2)	Stigma	+	-	-	-	-	-
		Ovule	-	+	-	+	-	-
c.	Mature unopened flower with 2/3 <sup>rd</sup> petal and 1/3 <sup>rd</sup> sepal (stage 3)	Stigma	+	-	+	-	-	+
		Ovule	-	+	+	+	+	-
d.	Mature open flower at anthesis (stage 4)	Stigma	+	+	+	+	+	+
		Ovule	+	+	+	+	+	+
Mature flower with fully opened banner petal (Stage 4)	6.	Total no. of ovules	3.3 ± 0.2	3.6 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	3.6 ± 0.1	3.9 ± 0.1
Fully formed Pod	7a.	Total number of seeds	2.8 ± 0.1	3.5 ± 0.2	2.7 ± 0.2	3.0 ± 0.2	3.4 ± 0.1	3.7 ± 0.2
	7b.	Number of seeds formed	2.1 ± 0.2	3.3 ± 0.2	2.3 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	2.5 ± 0.3
	7c.	No. of aborted seeds	0.7 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.8 ± 0.2	1.0 ± 0.3
	7d.	Seed abortion (%)	26.3	8.3	9.6	11.7	21.7	26.3
	8.	Seed : Ovule ratio	0.64	0.92	0.82	0.81	0.75	0.64
Mature flower with fully opened banner petal (Stage 4)	9.	Pollen:Ovule ratio	2557.57	2091.67	2553.57	2178.12	1961.11	1823.07

that pollen/pollination is not a limitation (Sengupta and Tandon 2010). Whereas the ovule number and the extent of its receptivity are under the genetic control of the species, optimization of brood size after fertilization becomes a function of net amount of resources available for grain filling.

Although the stigma in the cultivars Asha and Bahar became receptive at stage 2 or 3, the ovules appeared to be physiologically immature (non-receptive). In these taxa, the ovules attained receptivity only at anthesis. On the other hand in Pusa Ageti and T-21, the ovules became receptive first. In spite of this difference in the sequence of attainment of maturity in different parts of the pistil, all the cultivars exhibited ovular and stigma receptivity at anthesis. This suggests that the potential brood size (seed number per fruit) is optimized only at the time of anthesis. Such a correlation is in agreement with the other legumes investigated (Sengupta and Tandon 2010). In Upas 120, both the stigma and the ovules gain receptivity one stage prior to anthesis (stage 3). The number of mature seeds formed in a pod varied between 3 and 6. There was significant

difference among the cultivars in terms of total number of ovules and number of mature seeds formed (Wilks'  $\lambda = 0.557$ ,  $F_{(15,310)} = 4.875$ ,  $P = 0.001$ , MANOVA; Table 5; Fig. 2) but the seed abortion did not vary significantly. The total number of ovules and the total number of seeds is the maximum for the cv. Bahar, while the maximum number of mature seeds (non-aborted) are for the cv. T-21 followed by Pusa-33.

The seed to ovule ratio varied between 0.64 (Bahar and Asha) and ~0.92 (T-21). Although the cv. Bahar developed the highest total number of seeds, seed abortion was also maximum (26%) and this was similar to the abortion rate in the cv. Asha. Thus, in terms of seed production, T-21 was the leading seed producing cultivar amongst the six cultivars, followed by Pusa-33, Pusa Ageti, Bahar, Upas 120 and Asha (Fig. 2).

The amount of fruit-set differed significantly among the six cultivars ( $F_{(5,189)} = 48.29$ ;  $P = 0.001$ ; one-way ANOVA). Fruit-set was highest in cv. Pusa Ageti ( $5.85 \pm 0.22$ ) and Pusa-33 ( $4.19 \pm 0.19$ ) (Table 6). Also the fruit to flower ratio is

highest in Pusa Ageti and Pusa-33 cultivars viz. 0.55 and 0.47, respectively. The fruit-set per infructescence correlated with the amount of flower per inflorescence among the six cultivars ( $y = 0.525x + 7.34$ ;  $R^2 = 0.76$ ). The Pusa Ageti variety also exhibited greater pollen viability ( $86.6 \pm 1.4\%$ , Table 7) and low seed abortion (11.7%, Table 7) than the other varieties.

The preponderance of dichogamous protandry in the cultivars studied suggests the possibility of natural outcrossing, provided that a legitimate pollinator establishes floral constancy during the receptive phase of the flowers. According to Cruden (1977), the pollen: ovule ratio predicts a facultative xenogamous type of breeding system for the six cultivars of pigeonpea selected (Table 7). This clearly suggests that although the floral features are conducive for autogamy, there is immense possibility for outcrossing in the cultivars.

In insect-pollinated crops, floral biology in combination with functional floral morphology may exert a strong selection on pollination mechanism. In crop legumes the essential organs are concealed in the papilionaceous corolla even after maturity, but this may not guarantee successful autonomous selfing. Tripping is considered essential to facilitate pollen germination in legumes (Larkin and Graumann 1954), even if protandry results in deposition of self-pollen. Insect visits leading to tripping and the explosive dispersal of pollen from the vicinity of the keel petal is likely to facilitate mixed pollination. Tripping also leads to rupture of the thick cuticle-pellicle membrane to facilitate the release of stigmatic exudate (Bubar 1958), which otherwise remain unavailable for the germination of pollen grains (self or cross type). Enhanced visitation of pollinator in the crop is known to improve seed set (Sivaram and Jayaramappa 2013). Also, the earlier records on increased fruit-set through cross-pollinations than from selfing suggest the likely prevalence of inbreeding depression in pigeonpea (Choudhary *et al.* 2012, Sameer Kumar *et al.* 2012). The latter is yet to be established in the cultivars studied in the present work. In view of the above information, there appears to be ample scope for traditional breeding approaches to widen the genetic base of this genostatic crop. The baseline information provided on some of the cultivars of pigeonpea would prove handy in such experiments. In future, conventional breeding methods in combination with the genetic manipulation approaches may hold the key to break the static performance plateau of the crop.

**Acknowledgements**—DM is grateful to the Department of Science and Technology for the INSPIRE fellowship. RT is thankful for the financial assistance under DU-R&D Grant.

#### REFERENCES

- Abrams R 1967. Studies on natural cross-pollination in pigeonpeas (*Cajanus cajan*). *Puerto Rico Univ. J. Agr.* **51**(1) 1-3.
- Abrol DP 2012. *Pollination Biology: Biodiversity Conservation and Agricultural Production*. Springer, Dordrecht Heidelberg London New York.
- Aronne G 1999. Effects of relative humidity and temperature stress on pollen viability of *Cistus incanus* and *Myrtus communis*. *Grana* **38**(6) 364-367.
- Baker JR 1947. Further remarks on the histochemical recognition of lipids. *Q. J. Microsc. Sci.* **88** 463-465.
- Barros AF, Campos VP, da Silva JCP, Pedroso MP, Medeiros FHV, Pozza EA and Reale AL 2014. Nematicidal activity of volatile organic compounds emitted by *Brassica juncea*, *Azadirachta indica*, *Canavalia ensiformis*, *Mucuna pruriens* and *Cajanus cajan* against *Meloidogyne incognita*. *Appl. Soil Ecol.* **80** 34-43.
- Bhatia GK, Gupta SC, Green JM and Sharma D 1981. Estimates of natural cross-pollination in *Cajanus cajan* (L.) Millsp.: several experimental approaches. Proceedings of the International Workshop on Pigeonpeas, Volume 2, Patancheru, Andhra Pradesh, India 15-19 December 1980, Pp 129.
- Brar HS, Jhaji HS and Gatoria GS 1992. Abundance and activity of the bee visitors of pigeon pea (*Cajanus cajan* (L.) Millsp.) and role of *Apis mellifera* L. in its pollination. *Indian Bee J.* **54** 76-80.
- Bubar JS 1958. An association between variability in ovule development within ovaries and self-incompatibility in *Lotus* (Leguminosae). *Can. J. Bot.* **36** 65-72.
- Choudhary AK, Iquebal MA and Nadarajan N 2012. Protogyny is an attractive option over emasculation for hybridization in Pigeonpea. *SABRAO J. Breed. Genet.* **44** (1) 138-148.
- Cruden RW 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* **31** 32-46.
- Cumaraswamy A and Bawa KS 1989. Sex allocation and mating systems in pigeonpea, *Cajanus cajan* (Fabaceae). *Plant Syst. Evol.* **168**(1-2) 59-69.
- Dafni A, Kevan PG and Husband BC 2005 *Practical Pollination Biology*. Enviroquest Ltd., Ontario.
- Devasirvatham V, Tan DKY, Gaur PM and Trethowan RM 2014. Chickpea and temperature stress: an overview. In: Azooz MM & Ahmed P (eds.) *Legumes under environmental stress: yield, improvement and adaptations*. Wiley-Blackwell, New York Pp. 81-90.
- El Baradi TA 1978. Pulses 3. Pigeonpeas. *Trop. Agri. Abstr.* **4** 9-23.
- FAO 2012. Food and Agricultural Organization of the United Nation, FAO Statistical Database.

- Frankel R and Galun E 1977. *Pollination Mechanisms, Reproduction and Plant Breeding. Monographs on Theoretical and Applied Genetics 2*. Springer, Verlag Berlin Heidelberg New York.
- Grover A and Pental D 2003. Breeding objectives and requirements for producing transgenics for major field crops of India. *Curr. Sci.* **84** 310-320.
- Helsop-Harrison J and Heslop-Harrison Y 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technol.* **45** 115-120.
- IBM® SPSS® Amos™ 22 IBM Corp Released 2013. *Statistics for Windows, Version 22.0*. IBM Corp., New York.
- Johansen DA 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- Klinkhamer PGL and de Jong TJ 1990. Effects of plant size, plant density, and sex differential nectar reward on pollinator visitation in protandrous *Echium vulgare* (Boraginaceae). *Oikos* **57** 399-405.
- Krauss FG 1932. The Pigeon pea, (*Cajanus indicus*) its improvement, culture and utilization in Hawaii. *Hawaii Agri. Expt. Sta. Bul.* **64** 46.
- Kudo G and Harder LD 2005. Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Funct. Ecol.* **19**(2) 245-254.
- Lakshmi Sita G and Venkatachalam P 2008. Genetic transformation as a tool for improvement of pigeon pea, *Cajanus cajan* (L.) Millsp. In: Kirti PB (ed.) *Handbook of New Technologies for Genetic Improvement of Legumes*. CRC Press, USA Pp 125-145.
- Larkin RA and Graumann HO 1954. Anatomical structure of the alfalfa flower and an explanation of the tripping mechanism. *Bot. Gaz.* **116**(1) 40-52.
- Luo RH, Dalvi VA, Li YR and Saxena KB 2009. A study on stigma receptivity of cytoplasmic-nuclear male-sterile lines of pigeonpea, *Cajanus cajan* (L.) Millsp. *J. Plant Breed. Crop Sci.* **1**(6) 254-257.
- Mallikarjuna N and Saxena KB 2002. Production of hybrids between *Cajanus acutifolius* and *C. cajan*. *Euphytica* **124** 107-110.
- Mallikarjuna N, Jadhav D and Reddy P 2006. Introgression of *Cajanus platycarpus* genome into cultivated pigeonpea, *C. cajan*. *Euphytica* **149**(1-2) 161-167.
- Microsoft Office™ 2010. Microsoft Regional Sales Corp. Nevada, USA.
- Mir RR, Kudapa H, Srikanth S, Saxena RK, Sharma A, zam S, Saxena K, Varma Penmetsa R and Varshney RK 2014. Candidate gene analysis for determinacy in pigeonpea (*Cajanus* spp.). *Theor. Appl. Genet.* **127**(12) 2663-2678.
- Nepi M and Pacini E 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Ann. Bot.* **72**(6) 527-536.
- Pacini E 1992. Transport mechanisms of pollen—a short review. In: *Sexual plant reproduction*. Cresti M & Tiezzi A (eds.) Springer, Berlin Heidelberg pp. 69-79.
- Patel PT and Tikka SBS 2014. Gene action and stability parameters for yield and yield components, maturity duration and protein content of CGMS lines, pollen fertility restorers and their hybrids in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Euphytica* **199**(3) 349-362.
- Pearse AGE 1972. *Histochemistry, Theoretical and Applied (2<sup>nd</sup> edition)*. Churchill Livingstone, Edinburgh.
- Prasad S, Prakash R and Haque MJ 1977. Floral biology of pigeonpea. *Tropical Grain Legume Bull.* **7** 12.
- Purseglove JW 1968. *Tropical Crops: Dicotyledon*. Wiley, New York.
- Rao KN and Bramel PJ 2000. *Manual of Gene bank Operations and Procedures*. Technical Manual no. 6. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Sameer Kumar CV, Sreelakshmi C and Shivani D 2012. Gene effects, heterosis and inbreeding depression in Pigeonpea, *Cajanus cajan* L. *Electronic J. Plant Breed.* **3**(1) 682-685.
- Saxena KB, Singh L and Gupta MD 1990. Variation for natural out-crossing in pigeonpea. *Euphytica* **46**(2) 143-148.
- Saxena KB, Ariyanayagam RP and Reddy LJ 1992. Genetics of a high-selfing trait in pigeonpea. *Euphytica* **59**(2-3) 125-127.
- Saxena KB, Chauhan VS, Singh L, Kumar RV and Johnson C 1996. Research and development of hybrid Pigeon pea. *ICRISAT Research Bulletin* No. **119**.
- Saxena KB, Kumar RV, Srivastava N and Shiyng B 2005. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* **145**(3) 289-294.
- Saxena KB 2008. Genetic improvement of pigeon pea – a review. *Tropical Plant Biol.* **1** 159-178.
- Sengupta S and Tandon R 2010. Assessment of ovule receptivity as a function of expected brood size in flowering plants. *Int. J. Plant Repr. Biol.* **2** 51-63.

- Sharma K 1961. *Handbook of Agriculture*. Indian Council of Agricultural Research, New Delhi Pp 913-941.
- Shivanna KR and Rangaswamy NS 1992. *Pollen Biology (A Laboratory Manual)*. Springer-Verlag, Berlin Heidelberg.
- Singh L 1996. The development of and adoption prospects for extra-short duration pigeonpea. Pp 1–5 in Prospects for growing extra-short-duration pigeonpea in rotation with winter crops: proceedings of the IARI/ ICRISAT workshop and monitoring tour, 16–18 Oct 1995, New Delhi, India Singh L, Chauhan YS, Johansen C and Singh SP, (eds.). New Delhi, India: Indian Agricultural Research Institute; and Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Singh M, Gautam NK, Rana MK, Dahiya OP, Dutta M and Bansal KC 2014. Pigeon pea genetic resources and its utilization in India, current status and future prospects. *J. Plant Sci. Res.* **1** 107.
- Singh N, Tyagi RK and Pandey C 2013. *Genetic Resources of Pigeonpea (Cajanus cajan): Conservation for Use*. National Bureau of Plant Genetic Resources, New Delhi Pp 49.
- Singh RS, Singh APB and Singh DV 2015. Foraging behavior of the bee visitors to pigeon pea and their role in its pollination. *Progress. Agric.* **15(2)** 300-302.
- Sivaram V and Jayaramappa KV 2013. Influence of bee-attractants on pollination and yield in pigeon pea (*Cajanus cajan* (L.) Mill sp.). *Int. J. Plant Repr. Biol.* **5** 194-198.
- Srivastava RK, Isabel Vales M, Sultana R, Saxena KB, Kumar RV, Thanki HP, Sandhu JS and Chaudhari KN 2012. Development of 'super-early' pigeonpeas with good yield potential from early X early crosses. *J. SAT Agric. Res.* **10** 1-6.
- Taggar GK and Singh R 2015. Efficacy of some biopesticides against pod borer in pigeonpea. *Agric. Res. J.* **52(2)** 200-201
- Thomson JD 1988. Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evol. Ecol.* **2** 65-76.
- Upadhyay SK, Singh RP and Rizvi SMA 1997. Impact of bee pollination on the yield components of early variety of pigeon pea. National Symposium on Management of Biotic and Abiotic Stresses in Pulse Crops, IIPR, Kanpur, India.
- Vales MI, Srivastava RK, Sultana R, Singh S, Singh I, Singh G, Patil SB and Saxena KB 2012. Breeding for earliness in pigeonpea: development of new determinate and non-determinate lines. *Crop Sci.* **52** 2507-2516.
- Varshney RK, Graner A and Sorrells ME 2005. Genomics-assisted breeding for crop improvement. *Trends Plant Sci.* **10** 621-630.
- Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA, Donoghue MTA, Azam S, Fan G, Whaley AM, Farmer AD, Sheridan J, Iwata A, Tuteja R, Penmetsetsa RV, Wu W, Upadhyaya HD, Yang S, Shah T, Saxena KB, Michael T, McCombie WR, Yang B, Zhang G, Yang H, Wang J, Spillane C, Cook DR, May GD, Xu X and Jackson SA 2012. Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nat. Biotech.* **30** 83-89.
- Varshney RK, Roorkiwal M and Nguyen HT 2013. Legume genomics: from genomic resources to molecular breeding. *Plant Genome* **6** 1-7.
- Wani SP, Rockström J and Oweis TY 2009. Rainfed agriculture: unlocking the potential. Comprehensive Assessment of Water Management in Agriculture Series (7). CAB International, Wallingford, Oxon, UK Pp 310.
- Williams IH 1977. Behaviour of insects foraging on pigeon pea (*Cajanus cajan* (L.) Millsp.) in India. *Trap. Agric. Trinidad* **54** 353-356.
- Williams IH 1980. The pollination of pigeon pea (*Cajanus cajan* (L.) Millsp.) in India. Proceedings of the 2<sup>nd</sup> International Conference on Apiculture in tropical climates. New Delhi 29<sup>th</sup> March 1980.
- Wilsie CP and Takahashi M 1934. Natural Crossing in the pigeonpea. *J. Agr. Res.* **49** 923-927.
- Zheng-Hong LI, Liang N, Hong MA, Saxena KB, Tao Y, Xiu-Xian L and Xu-Xiao Z 2012. Insect pollinators in CGMS hybrid seed production of *Cajanus cajan*. *Acta Agronomica Sinica* **37(12)** 2187-2193.